ISSN 1346-7565 Acta Phytotax. Geobot. 63 (2): 99–105 (2013)

Potential Seawater Dispersal of Cypselas of *Sphagneticola trilobata* (L.) Pruski (Asteraceae), an Aggressive Invasive Alien Plant

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Sphagneticola trilobata (L.) Pruski (Asteraceae), considered to be among the world's worst invasive alien species, is indigenous to tropical America, but is now widely distributed in tropical and subtropical regions worldwide. In contrast to its vigorous vegetative reproduction, *S. trilobata* is thought to produce few fertile fruits. In this study, we investigated cypsela production in *S. trilobata* plants naturalized in the subtropical Ryukyu Archipelago, Japan, and assessed the viability of cypselas after exposure to seawater. Plants of *S. trilobata* produce an average of 7.8 cypselas per capitulum with a germination rate of more than 80%. Cypselas of *S. trilobata* showed high buoyancy in seawater and retained viability after exposure to seawater for 90 days. For preventive management of this species, potential seawater dispersal of cypselas, in addition to vegetative reproduction, should be considered.

Key words: conservation, exotic plant, fruit buoyancy, Ryukyu Archipelago, seed dispersal, seed production, Singapore daisy, *Wedelia trilobata*

Seed production and dispersability are two of many factors that affect the success of establishment of plant species. Data on these characteristics of invasive plants are extremely important for accurate risk assessment and effective management.

Sphagneticola trilobata (L.) Pruski (Asteraceae), formerly known as *Wedelia trilobata* (L.) Hitchc., but transferred to *Sphagneticola* O.Hoffm. by Pruski (1996), is a vigorous, creeping shrub native to tropical and subtropical America (Macoboy 2000, Fayaz 2011, USDA 2011). Because of its prolific production of brilliant yellow capitula throughout the growing season (Fayaz 2011), it has been planted for ornamental purposes in tropical and subtropical regions worldwide. Additionally, the creeping habit of *S. trilobata*, making it especially suitable for soil retention and erosion control, has further increased its artificial spread (Thaman 1999). In Japan, *S. trilobata* was successfully introduced to several regions, including the subtropical Ryukyu Archipelago (OECPMF 1997, Tachikake & Nakamura 2007), an assemblage of islands situated between Kyushu and Taiwan.

In many countries where *S. trilobata* has been introduced, it is recognized as a serious pest because it can cover other herbs and shrubs with a thick carpet of shoots, thereby shading and smothering indigenous plants and replacing them (Thaman 1999, ISSG 2005). Given its serious impact on native vegetation, *S. trilobata* is included among the world's 100 worst invasive alien species (Lowe *et al.* 2000). On many Pacific Islands, for example, this noxious alien plant is spreading in a wide variety of habitats, and its negative impact on the total ecosystem of the islands has been strongly affirmed (Thaman 1999).

Sexual reproduction in *S. trilobata* has been thought to be limited to only a few fruit (cypselas) per capitulum. For example, few cypselas mature in cultivated plants (Wagner *et al.* 1990),

fruits are not produced (Thaman *et al.* 1994, Yara & Kuba 2008), or plants are reported to spread only by asexual means (Meyer 2000). In contrast, because *S. trilobata* readily produces roots at the nodes of stems, it can reproduce quickly from cuttings resulting from periodic pruning (Thaman 1999). It is likely for this reason that transfer of vegetative organs is considered to have been a major contributor to the range expansion of *S. trilobata* (Thaman 1999). Because of relatively high fruit set in South America (Werpachowski *et al.* 2004), seed production in naturalized populations of *S. trilobata* should be re-evaluated. In addition, knowledge of seed dissemination is indispensable for risk assessment of invasive species. In particular, the presence or absence of long-distance dispersal of cypselas between islands, such as in the Ryukyu Archipelago and Pacific islands, will have a major effect on the management of *S. trilobata.* Fruit set and the dispersability of the cypselas of *S. trilobata* have never been studied intensively.

In this study, we investigated cypsela production in introduced *S. trilobata* in the Ryukyu Archipelago, Japan. We also tested the viability of cypselas after exposure to seawater for 90 days to assess the potential dispersal of cypselas by sea currents, which has been suggested in the preliminary observations on account of fruit morphology.

Materials and Methods

Fruit set analysis

Cypsela production per capitulum under open pollination was investigated in an ornamental planting of *Sphagneticola trilobata* at Senbaru, Okinawa Island, Japan (Table 1). The plants were located on a sunny bank about 200 m in length. A total of 128 capitula at anthesis were selected randomly in the population and marked with colored vinyl tape. The cypselas of *S. trilobata* are somewhat caducous, and are shed spontaneously from the capitulum as they mature, which results in inaccurate counts of mature cypselas. To overcome this problem, each of the randomly selected capitula was bagged with waterproof, breathable paper after all florets were drooping. Two to three weeks after bagging, all capitula were collected and the number of mature cypselas and drooping florets (both ligulate and tubular florets) were counted for calculation of fruit set in each capitulum.

In addition to open pollination, we evaluated the possibility of automatic self-pollination. A total of 98 capitula were selected randomly in the same population and bagged with waterproof, breathable paper before anthesis. The capitula were collected about four weeks after bagging and fruit set per capitulum was calculated by the same method as described above. Both the open pollination and bagging treatments were conducted from July to September, 2010.

Fruit buoyancy in seawater

Preliminary observations showed that the cypsela of *S. trilobata* have a spongy fruit wall (mesocarp) and a hard seed coat (Fig. 1), which are characteristics suggestive of water-dispersed fruits. Therefore, we conducted a buoyancy test

TABLE 1. Location of populations of *Sphagneticola trilobata* used in this study.

Island / locality	Latitude / longitude
Okinawa Island	
Hedo (Kunigami-son, Okinawa Pref.)	26°51′48.8″ N / 128°15′48.9″ E
Senbaru (Nishihara-cho, Okinawa Pref.)	26°15′7.5″ N / 127°46′4.1″ E
Hanashiro (Yaese-cho, Okinawa Pref.)	26°6′51.9″ N / 127°44′34.7″ E
Ishigaki Island	
Kabira-ishizaki (Ishigaki-shi, Okinawa Pref.)	24°28′39.9″ N / 124°6′57.2″ E
Iriomote Island	
Hoshidate (Taketomi-cho, Okinawa Pref.)	24°23′58.6″ N / 123°45′48.7″ E

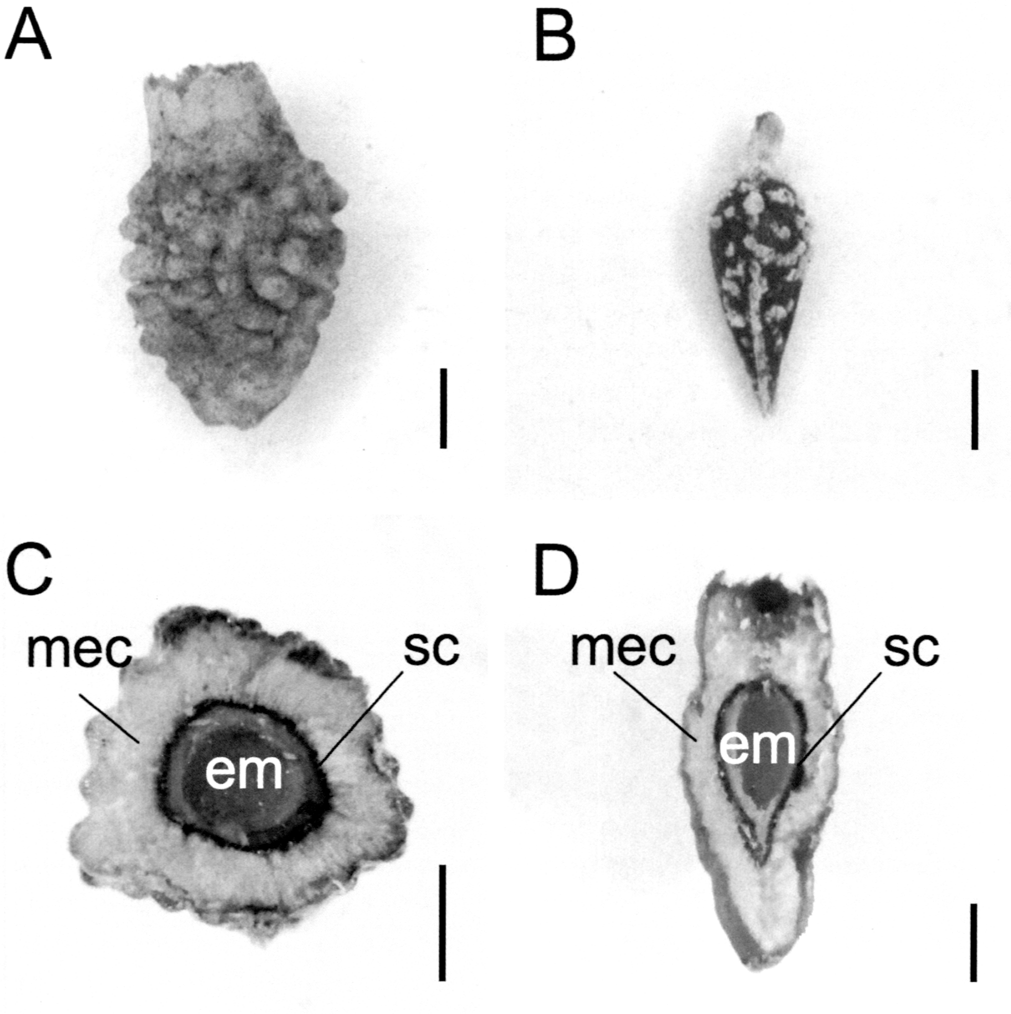

FIG. 1. Morphology of cypsela of *Sphagneticola trilobata*. A: Intact cypsela; B: cypsela with fruit wall removed; C: cross-section of cypsela; D: longitudinal view of cypsela. mec, mesocarp; em, embryo; sc, seed coat. Scale bars are 1 mm.

to evaluate the seawater dispersal ability of the cypselas of *S. trilobata*. Two-hundred and fifty cypselas were collected from each of five localities (Hedo, Senbaru, Hanashiro, Kabira-ishizaki, and Hoshidate) on Okinawa, Ishigaki and Iriomote islands (Table 1). *Sphagneticola trilobata* grows on coastal sandy beaches at Hedo and Hanashiro on Okinawa. At Kabira-ishizaki on Ishigaki and at Hoshidate on Iriomote, *S. trilobata* grows in wind-swept sites near the coast and in mangrove swamps. Part of the mangrove swamp at Hoshidate has been designated a conservation area, because it is at the northern limit of the range of *Acrostichum aureum* L. (Pteridaceae) (Yokota & Hiraiwa 2006).

Of the collected cypselas, 200 cypselas from each population were placed in separate plastic aquaria (W207×D132×H136 mm) half-filled with 1 L of seawater (salinity 3.3–3.5%), which was collected at Nakagusuku Bay in the southeastern part of Okinawa Island. The seawater in the aquaria was replaced with seawater every two weeks to maintain freshness. Sunken cypselas were counted every week during the experimental period (90 days) from 25 August to 25 November 2010. The experimental period (90 days) was decided following buoyancy tests in a preliminary study (Nakanishi 1988) in which a related species of *Wedelia* was treated. Considering the average velocity of the Kuroshio current is 89 cm per second (Bharatdwaj 2006), 90 days is sufficient time for floating disseminules to drift from

island to island within the Ryukyu Archipelago.

The remaining 50 cypselas collected from each locality were used as a control to test the viability of seeds not exposed to seawater. Each cypsela of *S. trilobata* contains a single seed, so intact cypselas were planted in the germination test. In a preliminary experiment, however, many untreated, fertile cypselas did not germinate after several months. For this reason, the fruit wall was removed before sowing and naked seeds were used to evaluate viability. The fruit wall was gently removed under a binocular microscope. The naked seeds were then sown on moist filter paper in Petri dishes and incubated at 25°C under a 12 h light/12 h dark photoperiod. The viability of seeds in cypselas that remained floating in seawater for 90 days was investigated in the same manner.

Results

In the open pollination treatment at Senbaru, the number of florets and mature cypselas per capitulum was 38.7 ± 10.2 and 7.8 ± 7.2 (average ± SD), respectively (Table 2). Fruit set per capitulum was 17.6 ± 15.4%. In the automatic self-pollination treatment, the number of florets and mature cypselas per capitulum was 41.4 ± 8.8 and 0.01 ± 0.1, respectively. Fruit set per capitulum was 0.03 ± 0.3% in this treatment.

Mature cypselas of *S. trilobata* were observed at all of the localities on Okinawa, Ishigaki, and Iriomote Islands. For example, many mature cypselas were produced at Kabira-ishizaki on Ishigaki Island, where *S. trilobata* formed a thick mat and was in the process of smothering native vegetation, including many endangered species such as *Chamaechrista garambiensis* (Hosokawa) Ohashi (Fabaceae) and *Sorghum nitidum* (Vahl) Pers. (Poaceae) (Fig. 2).

The results of a buoyancy test are shown in Table 3. Almost all cypselas from the five populations remained floating on the surface after 90 days exposure to seawater (average frequency of floating cypselas: 99.2 ± 1.2%). The percentage germination after 90 days exposure to seawater was 89.5%, 92.1%, 83.9%, 78.5% and 81.7% for seeds from Hedo, Senbaru, Hanashiro, Kabira-ishizaki and Hoshidate, respectively (average 85.1 ± 5.6%). The percentage germination of seeds without seawater exposure (control) was 86.7%, 81.7%, 80.0%, 83.3% and 85.0% for seeds from Hedo, Senbaru, Hanashiro, Kabira-ishizaki and Hoshidate, respectively (average 83.3 ± 2.6%).

Discussion

In the present research, fruit set in open pollinated plants of *S. trilobata* was 17.6%. Very low fruit set (0.03%) in the self-pollination treatment showed that fruit set in the absence of outcrossing was rare and much less frequent than in open pollinated capitula. The cypselas produced were viable and showed a relatively high germination frequency (more than 80%). Given that mature cypselas were frequently observed in many other localities on Okinawa, Ishigaki and Iriomote, viable cypselas are probably normal in *S. trilobata* in the subtropical Ryukyu Archipelago.

Werpachowski *et al.* (2004) reported 33.7 cypselas per capitulum in *S. trilobata* in Brazil, where the species is indigenous. The low frequency of 7.8 cypselas per capitulum at Senbaru indicates that fruit set in *S. trilobata* may vary greatly depending on the climate, environment and/or genetic structure of the population. The belief that *S. trilobata* does not produce viable cypselas (Thaman *et al.* 1994, Yara & Kuba 2008) is invalid in the Ryukyu Archipelago.

TABLE 2. Fruit set of *Sphagneticola trilobata* at Senbaru.

Treatment (no. of capitula)	Florets/capitulum	Cypselas/capitulum	Fruit set /capitulum (%)
Open pollination (128)	38.7 ± 10.2	7.8 ± 7.2	17.6 ± 15.4
Automatic self-pollination (98)	41.4 ± 8.8	0.01 ± 0.1	0.03 ± 0.3

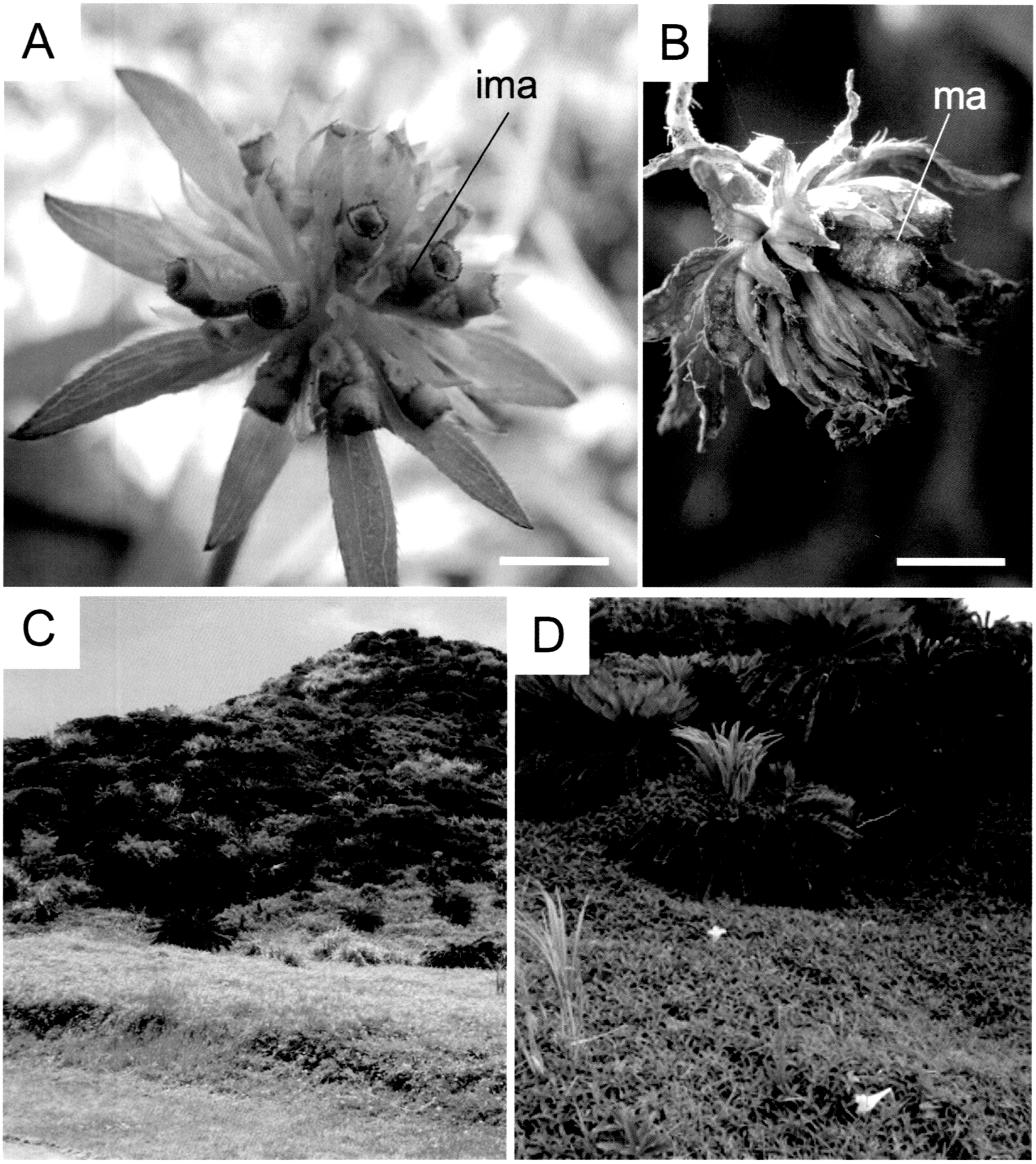

FIG. 2. Capitula with cypselas and growth habit of *Sphagneticola trilobata*. A: Capitulum with immature cypselas (ima). Ten cypselas were produced in this capitulum. B: Withered capitulum with mature cypselas (ma). C, D: Growth habit of *S. trilobata* at Kabira-ishizaki on Ishigaki Island. *Cycas revoluta* and many other indigenous plants were covered by a thick mat of *S. trilobata*. Scale bars in A and B are 5 mm.

TABLE 3. Buoyancy and germination frequency of cypselas of *Sphagneticola trilobata*

	90 days seawater exposure				Without seawater exposure (control)	
Locality	Number of cypselas tested	Number of floating cypselas	Floating cypselas (%)	Germination of floating cypselas (%)	Number of cypselas tested	Germination (%)
Hedo	200	200	100.0	89.5	50	86.7
Senbaru	200	200	100.0	92.1	50	81.7
Hanashiro	200	200	100.0	83.9	50	80.0
Kabira-ishizaki	200	197	98.5	78.5	50	83.3
Hoshidate	200	195	97.5	81.7	50	85.0
Average	200	198.4 ± 2.3	99.2 ± 1.2	85.1 ± 5.6	50	83.3 ± 2.6

As mentioned by Thaman (1999), *S. trilobata*, which has very wide ecological tolerances, spreads uncontrollably in a variety of habitats and often forms large, dense infestations. Such occurrences appear to be relatively common on many islands in the Ryukyus. Considering that *S. trilobata* blooms throughout the year except in the coldest seasons (OECPMF 1997) and produces an average of 7.8 cypselas per capitulum, total cypsela production may be too high to be ignored. Furthermore, mature cypselas had a high germination frequency of more than 80%. Reproduction by seed in naturalized *S. trilobata* could therefore make a notable contribution to its further spread. Wanger *et al.* (1990) stated that, "If a fertile strain develops this species could become a serious pest." Our results demonstrate that Wanger's comment is not mere speculation, but a reality that must be considered by conservation authorities.

Fruit also plays an important role in plant dispersal. Fruits endowed with specialized structures can enable plant species to disperse over long distances with the aid of wind, birds and sea currents. The cypselas of *S. trilobata* in seawater showed a floating ability of nearly 100% over a 90 days period. This buoyancy is almost identical to that reported in other typical maritime plants, such as *Wedelia biflora* (Nakanishi 1988). It is therefore assumed that *S. trilobata* has potential to be dispersed by sea currents via its cypsela. In addition, cypselas of *S. trilobata* retain a high germination frequency of about 85% after 90 days exposure to seawater. This percentage is much higher than in *Wedelia biflora* (54% after three months of exposure to seawater) (Nakanishi 1988). Our findings further support the potential for seawater dispersal of *S. trilobata*. Similar to the many other countries where *S. trilobata* has been introduced, this vigorous plant frequently occurs in coastal habitats on islands (T. Denda, pers. obs.). Cypselas of *S. trilobata* in such habitats have a greater opportunity to be swept away and be dispersed by ocean currents.

Thaman (1999) mentioned that rapid spread of *S. trilobata* was due to easily-established cuttings being transported to waste places, dumps (rubbish tips) or dumped along seashores or riverbanks, or thrown into the water, where they easily establish themselves and can be carried by rivers and streams, or even by ocean currents, to other potential sites. Thaman (1999) strongly demanded the eradication of *S. trilobata* especially from coastal environments. Our present findings on the potential for dispersal of cypselas by sea currents substantiates the comments of Thaman.

To prevent invasion by *S. trilobata* requires the prohibition of its planting and the removal of existing plants. Once introduced, artificial transfer by dumping of vegetative organs, such as cuttings, should be prohibited. Most preventive management programs consider these points, but less attention seems to

have been paid to cypsela dispersal by sea currents. The present finding indicate that sexual reproduction and seed dispersability of *S. trilobata* has been underestimated and these factors should be taken into consideration for future risk assessment and preventive management of the species.

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Received July 19, 2012; accepted August 8, 2012